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Himalayan 'yeti' DNA: polar bear or DNA degradation? A comment on 'Genetic analysis of hair samples attributed to yeti' by Sykes *et al*. (2014)

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Based on 104 base pairs of the mitochondrial 12S ribosomal RNA gene, Sykes *et al*. [1] identified two Himalayan samples of hair, from Ladakh (India) and Bhutan, respectively, as belonging to the species *Ursus maritimus* (polar bear). The authors claim that these samples have the closest genetic affinity to a Palaeolithic polar bear from Spitsbergen, Svalbard [2]. We contest the identification of these samples on several grounds.

The first issue we refute is that the two sequences, derived from hair samples nos 25025 and 25191, have 'a 100% match with DNA recovered from a Pleistocene fossil more than 40 000 BP of *U. maritimus* (polar bear) [2] but not to modern examples of the species'. This is an incorrect statement. Hairs 25025 and 25191 have a 100% identify with GenBank accession no. GU573490, which belongs to a modern *U. maritimus* individual from Diomede, Little Diomede Island, Alaska (isolate 495) [2]. The Pleistocene polar bear, GU573488 (P), has one transitional difference from this sequence at position 1675 (using the numbering from the polar bear NCBI reference sequence NC_003428 [3]; figure 1).

We reanalysed the 10 *Ursus* sp. sequences generated in Sykes *et al*. [1] and compared them with data deposited in GenBank. We undertook a BLAST search using KJ155697 (hair 25025) as the search query and downloaded all accessions with 100% query cover (i.e. where the full 104 base pair (bp) fragment was covered) and which had greater than 95% identity. This generated 155 modern and 28 ancient sequences as follows: 111 brown bear (*U. arctos*); 32 polar bear (including one ancient [2]); eight Asian black bear (*U. thibetanus* sp.); three American black bear (*U. americanus*); 26 extinct cave bear (*U. spelaeus*); one extinct Deninger's bear (*U. deningeri*) and two sloth bear (*Melursus ursinus*). We constructed a network (figure 1) by use of a median-joining algorithm [4] as implemented in the NETWORK 4.612 program (www.fluxus-engineering.com/sharenet.htm). The 'diagnostic' base change between the majority of the GenBank bears and the hairs 25025 and 25191 plus accession GU573490 (denoted as haplotype A in figure 1) is a transition at position 1751. This mutation occurs in a stretch of C bases near the 3′ end of the sequence, at position 100 of the 104 bp fragment. All 36 polar bear from GenBank, including the Pleistocene individual (P), have a T base at this position, although, as can be seen in figure 1, a number of brown bear also have a T. Other bears either have a C base or a deletion. The brown bears that share the T mutation with the Sykes *et al*. [1] 'polar bears' include five modern bears from the ABC Islands off Alaska (GU573486, GU573487, GU573489, JX196368 and JX196369 [2,5]; haplotype D in figure 1). This is unsurprising as the ABC brown bears share a matrilineal common ancestor with polar bears around 24 000 years ago [6]. However, the other three bears are modern samples originating from Europe, namely the Balkan Mountains in Bulgaria (AP012591 [7]), the Guzet shelter in Ariège, France (EU497665 [8]) and an unspecified location in Europe (U12854 [9]). These brown bears group with 31 modern polar bears, as well as with the Pleistocene polar bear sequence (P in figure 1).

It is noted that the hair shaft samples analysed by Sykes *et al*. [1] covered a wide range of ages and conditions, ranging from fresh to museum specimens

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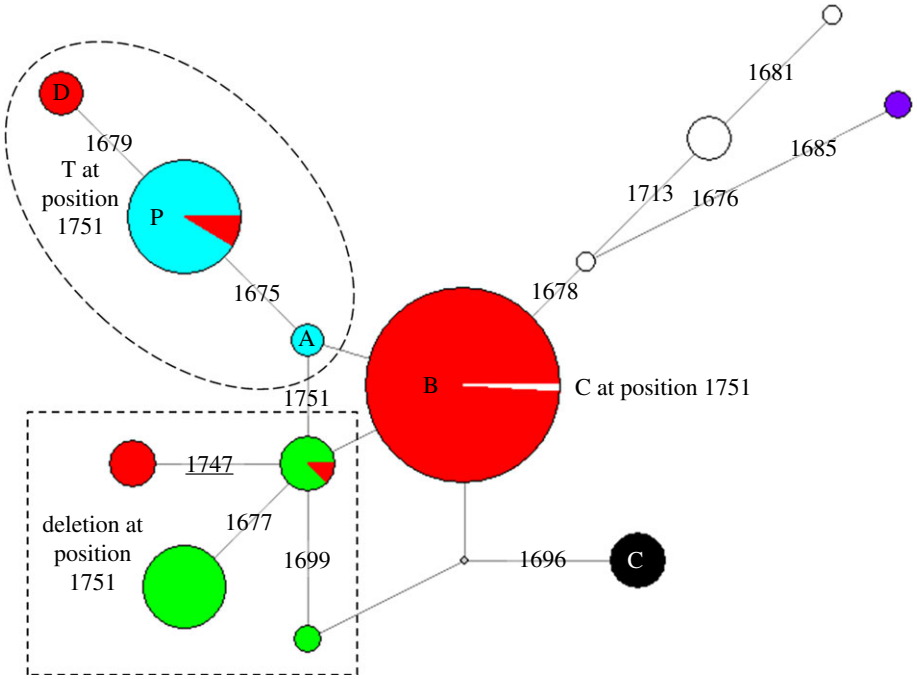

Figure 1. Median-joining network analysis of the 104 bp region of the of the mitochondrial 12S ribosomal RNA gene. The colours denote which species the samples belong to: turquoise, polar bear; red, brown bear; black, American black bear; white, Asian black bear; green, cave bear (plus one Deninger's bear); purple, sloth bear. For information on sample sizes, see main text. Circle areas are proportional to the number of individuals possessing each mitochondrial haplotype. Named haplotypes are as follows: A, hair samples 25025 and 25191, plus one modern polar bear (GU573490); B, hair samples 25027 and 25194, plus 96 brown bear from GenBank and one Asian black bear (AB302321); C, hair samples 25028, 25074, 25082, 25104, 25106 and 25202, plus three American black bear (AF303109, JX196366 and Y08520); D, five brown bear from the ABC Islands (see main text for accession numbers). The position of the Pleistocene polar bear GU573488 [2] is noted by a P and is one transition away from haplotype A. The positions of the nucleotide substitutions that distinguish the haplotypes are shown, using the numbering from the polar bear NCBI reference sequence NC_003428 [3]. All mutational differences are transitions, apart from 1747 (underlined), which is a transversion. One unsampled intermediate node is demarked by a small point.

more than 50 years old. Sample no. 25025 came from a group of hairs collected over 40 years ago, whereas the age of sample no. 25191, a single hair, is not documented. The fact that these samples are not modern technically makes them historic DNA, which is a sub-group of ancient DNA. Ancient DNA is any DNA that has undergone autolytic or diagenetic processes, such as oxidation (which causes chemical modification of sugar residues and pyrimidine bases), endogenous hydrolysis (which occurs rapidly after death and breaks phosphodiester and glycosidic bonds), breakage of strands and formation of cross-links [10]. These damages may prevent useful sequence data being obtained or can, in some instances, give false results.

This being said, hair is generally considered to be a good source of ancient DNA as it can be decontaminated easily to remove exogenous DNA [11]. Sykes *et al*. [1] state that the hair shaft samples were thoroughly cleaned to remove surface contamination, but they do not go into details as to what this cleaning entailed. The diagnostic mutation that separates modern brown bears from modern polar bears occurs at position 1751 (figure 1) and, as mentioned, is a C/T transition. This base change sits in the middle of a run of six C bases, which is exactly the place to expect a damage artefact. Deamination of C (cytosine) to U (uracil) is the major mechanism leading to miscoding lesions in ancient DNA [10] and causes C to T substitutions. One way to determine whether the T base seen in hairs 25025 and 25191 is real, and not an artefact of DNA degradation, would be to impose the basic criteria for analysis of ancient DNA (e.g. [12]). While we understand that replication of the samples may be impossible (especially due to 25191 being from a single hair), the direct PCR results should have been verified by cloning of the amplified products, which would determine whether the C/T mutation is correct and not due to a damage-induced error in sequencing. Additional PCRs could also have been attempted from the extracts to build a consensus sequence for each sample. We would contend that this should have been standard practice when dealing with a controversial result, such as that reported by Sykes *et al*. [1].

Assuming that the single-base change that made Sykes *et al*. [1] assign the hairs 25025 and 25191 as polar bear is a damage artefact, we should like to propose an alternative origin for the hair samples from Ladakh and Bhutan. The Himalayan bear (*U. arctos isabellinus*) is a sub-species of the brown bear that lives in the higher reaches of the Himalayas (*ca* 3000 to 5500 m), in remote, mountainous areas of Pakistan, Nepal, Tibet, Bhutan and India. Its populations are small and isolated, and it is extremely rare in many parts of its range [13]. The common name for these bears in the region is *Dzu-teh*, a Nepalese term meaning 'cattle bear', and they have long been associated with the myth of the yeti [14]. They are usually sandy or reddish-brown in colour. As the two hair samples tested by Sykes *et al*. [1] were golden-brown (Ladakh, 25025) and reddish-brown (Bhutan, 25191), and as the most parsimonious explanation of the sequences recovered is that they came from brown bear and exhibit DNA degradation, we would contend that the hair samples are, in fact, from Himalayan brown bears and not from 'a previously unrecognized bear species, colour variants of *U. maritimus*, or *U. arctos*/*U. maritimus* hybrids' as claimed.

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